## REMARKS

Subsequent to the filing of the Appeal Brief, the Examiner has reopened prosecution of the present application and has issued an Official Action dated July 29, 2003. In the Office Action, claims 18-20, 27 and 28 are rejected under 35 U.S.C. §103(a) as allegedly obvious over Gorman in view of Builder et al. (U.S. Patent 5,663,304), Meulien (U.S. Patent 5,521,070), Ritter et al. (*J. Biological Chemistry* 266: 1043-1047, 1991) and Ciotti et al. (*Biochemistry* 5: 10119-10124, 1996). Claims 25-26 and 29-31 are rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enabling support. The Examiner states that Appellant must file a reply under 37 C.F.R. § 1.111 or request a reinstatement of the appeal in order to avoid abandonment of the application.

In response to the Official Action and in accordance with the provisions of 37 C.F.R. §§1.111 and 1.121, Applicants respectfully submit the instant amendment for entry in the above-identified case. This amendment addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claims 18-20, 27 and 28 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Gorman in view of Builder et al., Meulien, Ritter et al. and Ciotti et al.

In an effort to favorably advance the prosecution of the present application, Applicants have canceled claims 18-20 and 27-28 without prejudice by way of the instant amendment. Applicants reserve the right to pursue the subject matter of these canceled claims in a continuation application. As such, the rejection of claims 18-20 and 27-28 under 35 U.S.C. §103(a) is rendered moot. Withdrawal of the rejection is therefore respectfully requested.

Claims 25-26 and 29-31 are rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enabling support. It is observed that claims 25-26 and 29-31 are drawn to Sertoli cells comprising a vector that encodes a biological factor, wherein said Sertoli cells create an immunologically privileged site *in vivo*.

The Examiner contends that the claimed invention involves gene therapy wherein a Sertoli cell transfected *ex vivo* with a construct encoding a heterologous protein is used to supplement a deficiency in a subject *in vivo*. Referring to Verma et al. (1997), the Examiner contends that gene therapy is associated with various problems and unpredictability. Thus, the Examiner concludes that, based on the state of the art at the time the invention was made and absent evidence to the contrary, the expression of the biological factor from the transformed Sertoli cells *in vivo* would be unpredictable. In addition, the Examiner contends that the claimed invention involves the creation of an immunologically privileged site such that the transplanted cells are not affected by the subject's immunological responses. The Examiner contends that it is difficult to extrapolate the function of Sertoli cells in making the testis an immunologically privileged site to creating an immunologically privileged site anywhere in the body of a subject. The Examiner states that the specification does not provide any data showing that the biological factor is expressed in the subject or that the Sertoli cells create immunologically privileged sites *in vivo*. Moreover, the Examiner contends the kidney sub-capsular space, which is taught in the specification as site for implantation of transfected Sertoli cells, is itself an immunologically privileged site. Therefore, the Examiner concludes that, absent further evidence and in view of the unpredictability of the art, it would take undue experimentation for those skilled in the art to practice the claimed invention.

In the first instance, Applicants respectfully submit that claims 25-26 and 29-31 simply recite a Sertoli cell "comprising" a vector. Although the claimed Sertoli cells can be obtained by transfecting or transforming isolated Sertoli cells, the claims do not require the Sertoli cells to be transformed *ex vivo*, or require transformation to be the only means for obtaining genetically altered Sertoli cells. As described in the specification, e.g., on page 6, second paragraph, Sertoli cells used to produce the biological factor can be generated by either *ex vivo* gene transfer or isolated from a transgenic animal that expresses the biological factor in Sertoli cells. To more clearly delineate this feature of the present invention, Applicants have added claim 32 to recite that the Sertoli cell is isolated from a transgenic animal, and wherein the transgenic animal comprises the vector coding for the biological factor and expresses the biological factor.

Applicants respectfully submit that the present specification provides adequate guidance for those skilled in the art on how to make and use the Sertoli cells, as presently claimed. Specifically, Sertoli cells can be isolated from an animal, including a transgenic animal, by employing the methods described in the specification, e.g., at pages 13-14. Isolated Sertoli cells can be transfected with a vector that encodes the desired biological factor, as described at page 23, line 18 through page 24, line 22. Alternatively, transfection may not be necessary if Sertoli cells are isolated from a transgenic animal that has been genetically engineered to express the desired biological factor. As described in the last full paragraph at page 7, a transgenic animal that expresses a biological factor in Sertoli cells can be made by using a number of methods known to those skilled in the art, including the standard procedure of microinjection into pronuclei. In this connection, Applicants observe that transgenic mice, rats, rabbits, pigs, sheep and cows have been produced, including transgenic animals that express human growth

hormone and Factor VIII, as evidenced by Murphy et al. ("*Transgenesis Techniques: principles and protocols*", Totowa, NJ: Humana Press, Inc.; 1993), Pinkert ("*Transgenic animal technology: a laboratory handbook*", San Diego, CA: Academic Press, Inc., 1994), Hiripi et al. ("*Expression of active human blood clotting factor VIII in mammary gland of transgenic rabbits*", *DNA Cell Biol.* 22:41-45, 2003; **Exhibit 1**), Niemann et al. ("*Expression of human blood clotting factor VIII in the mammary gland of transgenic sheep*", *Transgenic Res.* 8:237-241, 1999; **Exhibit 2**), and Lipinski et al. ("*Transgenic rabbit producing human growth hormone in milk*", *J. Appl. Genet.* 44:165-174, 2003; **Exhibit 3**).

Applicants further respectfully submit that the specification teaches how to achieve expression of a desired biological factor in Sertoli cells *in vivo*, for example, by placing the coding sequence of the biological factor under control of a promoter which functions in Sertoli cells. See the paragraph bridging pages 16-17 of the specification. The Sertoli cells can be transplanted into an appropriate site in a recipient animal to achieve the expression of the biological factor *in vivo*. See the middle paragraph at page 26 of the specification.

In this regard, Applicants provide herewith a report by Wayne et al. ("*Expression of the Pem homeodomain gene in Setoli cells increases the frequency of adjacent germ cells with deoxyribonucleic acid strand breaks*", *Endocrinology* 143:4875-4885; 2002, attached hereto as **Exhibit 4**). The report demonstrated the generation of transgenic mice in which the homeobox protein Pem was expressed in Sertoli cells. Pem is a transcription factor normally found transiently in Sertoli cells. Thus, the results shown by Wayne et al. indicate that Pem transgenic animals could be generated using the Sertoli cell-specific Pem promoter with Pem expression selectively in Sertoli cells throughout all stages of development.

As further support of the enablement of the claimed subject matter, Applicants provide herewith a declaration of Dr. Craig Halberstadt (attached hereto as **Exhibit 5**), one of the co-inventors whom Applicants have sought to add as a co-inventor by a Petition to Correct Inventorship, filed on August 8, 2002. In the declaration, Dr. Halberstadt describes experiments conducted in his laboratory as well as his collaborators at the University of Alberta in Edmonton, Canada, in which Sertoli cells were isolated from transgenic mice that were engineered to produce green fluorescent protein (GFP). The isolated Sertoli cells (expressing GFP) were transplanted under the kidney capsule of immunocompromised SCID mice as well as allogeneic, immunocompetent Balb/c mice. The results show that the Sertoli cells survived the transplantation into both the SCID mice and the Balb/c mice, and expressed the foreign protein GFP in the recipient mice. Hence, the Sertoli cells were able to protect themselves in an allogeneic environment and were able to express a foreign protein.

With respect to the ability of genetically altered Sertoli cells to create immunologically privileged sites *in vivo*, the Examiner contends that the kidney sub-capsular space which is taught in the specification as a site for transplanted Sertoli, is itself an immunologically privileged site.

In the first instance, Applicants wish to point out that the Examiner's characterization of the kidney sub-capsular space as an immunologically privileged site, which is apparently based solely on the abstract of Cole et al. (*J. Materials Science: Materials and Medicine* 4: 437-442), is incorrect. Applicants are providing a courtesy copy of Cole et al. in full text for the Examiner (**Exhibit 6**). Applicants observe that the only place in Cole et al., where the phrase "immunologically privileged site" is used to describe the kidney subcapsular space, is in the abstract of the paper. The actual content of Cole et al. does not support such a characterization.

Specifically, the condition of encapsulated islets implanted in the peritoneal cavity was compared to those implanted in the kidney subcapsular space (see section 3.2. of the Results section). If the kidney subcapsular space were an immunologically privileged site, the encapsulated islets and the surrounding tissue would not be rejected. To the contrary, Cole et al. observed a "marked reaction" (in caption of Figure 1) and reported the presence of "fibroconnective tissue together with inflammatory cells", indicating an immunological response to the grafted tissue in the kidney subcapsular space.

Applicants wish to point out to the Examiner that other investigators have also used the kidney subcapsular space as a site in which to transplant islets. For example, Selawry and Cameron (Cell Transplantation 2:123-9, 1993; **Exhibit 7**) reported the transplantation of allogeneic islets under the kidney capsule of PVG rats. Notably all islet transplants of islets alone failed to produce functional grafts, demonstrating that the kidney subcapsular space is not immunologically privileged. Only when exogenous immunosuppression was added (or notably the addition of Sertoli cells to the transplant) did the grafts succeed.

Further regarding the ability of genetically altered Sertoli cells to create immunologically privileged sites *in vivo*, the Examiner also contends that it is difficult to extrapolate the function of Sertoli cells in making the testis an immunologically privileged site to creating an immunologically privileged site anywhere in the body of a subject.

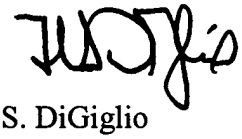
In this regard, Applicants respectfully submit that, prior to the filing of the present application in 1998, non-transgenic Sertoli cells had been shown to create immunologically privileged sites *in vivo* to protect islets and neuronal cells from immune-mediated rejection. See, for example, Selawry et al. (**Exhibit 7** above), and Sanberg et al. ("*Testis-derived Sertoli cells survive and provide localized immunoprotection for xenografts in rat brain*", *Nat Biotech* 1996;

14: 1692-1695; **Exhibit 8**). Further, Applicants respectfully direct the Examiner's attention to the study described in Dr. Halberstadt's declaration. The fact that transgenic Sertoli cells survived as allografts in recipient mice indicates that the expression of a foreign protein, which is immunogenic itself, did not interfere with the ability of Sertoli cells to protect themselves against immune rejection. Thus, these results certainly support the ability of genetically altered Sertoli cells to create immunologically privileged sites *in vivo*, as presently claimed.

In view of the foregoing, Applicants respectfully submit that the claims are fully supported by the specification and that those skilled in the art would be able to make and use the claimed Sertoli cells without undue experimentation. As such, it is respectfully submitted that the rejection under 35 U.S.C. §112, first paragraph, is overcome. Withdrawal thereof is respectfully requested.

Accordingly, it is believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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Encl: Exhibits 1-8.